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TOXAPHENE

TESTING PROCEDURE FOR EMULSIFIABLE CONCENTRATES OF TOXAPHENE

ADMINISTRATIVE

U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

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TESTING PROCEDURES FOR EMULSIFIABLE CONCENTRATES OF TOXAPHENE

PURPOSE

This bulletin is concerned with toxaphene emulsifiable concentrates as applied to official permitted dips for the treatment of scabies infected cattle and sheep. It does not undertake to discuss toxaphene formulations intended for other applications or subject to other criteria. The procedural information and technical data presented are intended primarily to aid the manufacturer in the preparation of a stable and effective acaricidal emulsion dip formulation. This bulletin should also serve as a guide for the evaluation of toxaphene formulations by official agricultural disease control and other laboratories.

Regulations and laws pertinent to scabies control in cattle and sheep are given in the Code of Federal Regulations, Title 9, Chapter I, Subchapter C, Parts 73 and 74. The Animal Disease Eradication Division of the Agricultural Research Service is administrator for these regulations. As such, this Division must necessarily reserve the power to prescribe the chemical content, physical performance, and field application and use procedures for substances employed in its disease eradication programs.

INTRODUCTION

Toxaphene is a chlorinated hydrocarbon produced by chlorinating camphene, commercially obtained from southern pine, to the extent of 67 to 69% chlorine. It has an average empirical formula of $C_{10}H_{10}Cl_8$, and a corresponding molecular weight of 414. It was introduced for insecticidal purposes in 1947 (26). Formulated as a 61% by weight emulsifiable concentrate, it is widely used for the control of external parasites on cattle,

sheep, hogs, goats and horses. The formulation consists of solvent plus emulsifier, or mixture of emulsifiers, and toxaphene at 6.1 pounds per 10.0 pounds of concentrate. The specific gravity of the commonly used concentrates is in the range of 1.23.

The emulsion concentrate is designed to be diluted with water to provide a 0.5% solution of toxaphene which is then applied to livestock using power sprays or large vat dips. Livestock concentrates should exclude ingredients which might be harmful to animals. They must be formulated to yield exceptionally stable emulsions.

It is no easy matter to obtain an emulsifiable concentrate which will be stable under the many parameters impinging on the product's performance in the field. An emulsion stability test herein described is based upon extensive field studies of dip stability and is accepted by the Animal Disease Eradication Division as an adequate laboratory guide to the stability of an emulsion under field conditions. The manufacturer should ascertain that his formulation will comply with this test before attempting to have his product added to the list of permitted dips.

There are no restrictions regarding the solvent or emulsifiers to be used in toxaphene emulsifiable concentrates so long as these are non-injurious to livestock under normal conditions of use and otherwise meet program requirements. The active ingredient, toxaphene, must, however, be maintained within a very limited range around 61% by weight concentration. Spectrophotometric and chemical analytical methods are described in this paper that will enable determination of toxaphene concentration in emulsifiable concentrates.

Assuming that a given formulation is acceptable as a permitted, official, acaricidal dip, it is next necessary that this product be properly used in the field. As animals are dipped, the concentration of toxaphene is diminished in a proportion to the number of animals processed, dependent on a variety of factors, such as length of hair or wool, condition of coat, etc. The vat, then, must be replenished with fresh concentrate at given intervals such that every animal is exposed to an approximately 0.5% toxaphene concentration. Lower concentrations would not yield an effective acaricidal dip, and higher concentrations may result in toxic effects to livestock.

Maintaining livestock vat dips at a constant concentration of 0.5% toxaphene requires special handling and strict adherence to vat management procedures, once these have been established. The Animal Disease Eradication Division issues detailed instructions for the guidance of employees using toxaphene emulsion dips in the scabies eradication program. These instructions should be scrupulously followed.[1]

Unfortunately, at this writing, no acceptable field vat-side test for toxaphene dip concentration has been developed. Therefore, it will be necessary for the field worker to call upon the chemical analytical laboratory to accurately and rapidly check his vat management technique. For this purpose, simple and rapid spectrophotometric and chemical analytical methods for toxaphene in livestock emulsion dips are described herein.

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1. Inquiries should be directed to Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Federal Center Building, Hyattsville, Maryland 20781.

FUNDAMENTAL REQUIREMENTS FOR TOXAPHENE EMULSIFIABLE CONCENTRATES

In order to establish criteria for toxaphene emulsifiable concentrates it is first necessary to review briefly the function such a formulation is expected to perform: Ideally, a toxaphene concentrate, when diluted with water, should present a stable, uniform, homogeneous bath to an animal's body surface, which will result in the destruction of all scabies mites, in various stages of life cycle, present on this surface without causing injury or harm to the animal. Providing a concentrate is properly formulated, this ideal is not incapable of approach.

Perhaps the least difficult variable capable of control is the quantity of toxaphene presented to the animal's surface per gross, or milliliter, unit of bath volume. However, it must be remembered that on a micro scale, the acaricide is presented not as a homogeneous solution but as an emulsion, which is a heterogeneous system of two (or more) phases. In such a system, areas or particles of organic phase containing toxaphene are separated by an aqueous phase, essentially free from toxicant. Thus, a non-uniform, non-homogeneous acaricidal bath is in contact with the animal's body.

The approach to ideality is lost, particularly as the size of the particles of the two phases is increased. When the toxicant phase is more finely dispersed, greater surface area of toxicant is presented; conversely, with increasing diameter of emulsion particles, less toxicant surface is exposed to the animal. Thus, the availability of toxaphene for acaricidal purposes is primarily a function of emulsion particle size.

Further, as the particle size of organic phase containing toxaphene increases, the image of the animal body as presenting a smooth surface to toxicant is lost. It has been shown (29) that large or agglomerated emulsion particles are filtered from emulsion by the animal's wool or hair at a much greater rate than are smaller particles. Thus, animals may strain out high concentrations of pesticide from dips containing many coarse emulsion particles but with apparently safe concentration of toxaphene. This has been a cause of pesticide toxicity to livestock (34).

We have seen that to a large extent, the availability of toxaphene and, therefore, its acaricidal effectiveness and toxicity to livestock is a function of the dispersion or fineness of the emulsion formed upon addition to water. It is imperative, therefore, that a concentrate will yield small emulsion particles upon dilution with water (sizes well below 1.5 microns in diameter) and that these particles will retain their size for long periods.

The aqueous emulsion stability is the time measured ability for an emulsion to retain the same fine emulsion particle size. It is primarily dependent on the proper choice of solvent and emulsifier.

For purposes of definition, it may be said that when an emulsion, for any of a number of reasons, forms particles large enough to cause agglomeration with consequent separation from suspension, this emulsion is unstable at that point in time. Such separations are variously des cribed as "breaking", "creaming", "settling-out", "oiling-out", etc., depending on individual symptoms. Causes for deterioration of dip emulsions are many and varied. They include factors such as the number

and the character of ions present in the water employed (water hardness), the water temperature, the amount and the nature of materials contributed by the livestock dipped, weather conditions, vat construction, methods employed in preparing the emulsion dip, etc.

Obviously, it will be impossible to control the many variables operating in the field which are antagonistic to an emulsion's stability. However, it should be possible to formulate a concentrate which will obtain such exceptional stability as to successfully overcome all or most of the difficulties encountered. This bulletin describes a test for emulsion stability which has been empirically determined to encompass most field conditions. If a product will pass this test in the laboratory, it will usually meet with the problems discussed in this section.

PHYSICAL TEST FOR EMULSION STABILITY

This test is a modification of a method proposed by J. C. Clark, et. al. (6) and is based upon extensive field studies of dip stability. The ability of a toxaphene emulsion concentrate to pass this test is taken to be an acceptable measure of suitability for use in livestock vat dips providing the formulation contains the proper concentration of pesticide.

Apparatus:

1) Tubing, Pyrex glass, 4 feet long by 22 mm outside diameter, Corning #234220, or equivalent. Fifteen are required per test.

2) Conical centrifuge tubes, 15 ml graduated, Corning #8080, or equivalent. Fifteen are required per test.

3) Rubber tubing, amber latex, 5/8 inch bore, 1/8 inch wall, The Chemical Rubber Company Cat. #13-8910, or equivalent.

An emulsion test tube is constructed from the above materials by attaching the beaded lip of a conical centrifuge tube flush to the end of the Pyrex glass tube by means of an approximately $2\frac{1}{2}$ inch piece of rubber tubing.

4) "Luer-lok" syringe, 5 cc capacity, Becton, Dickinson and Company, Cat. #H5WL, or equivalent, fitted with a 4 inch 13 gauge stainless steel blunt end needle bent at about 90° approximately 2 inches from its end.

Reagents:

1) Distilled water.

2) Soft water with hardness equivalent to 20 parts per million calcium carbonate. It is composed of 0.0094 grams of calcium chloride, 0.8000 grams of sodium bicarbonate and 0.0103 grams of magnesium chloride per liter of distilled water.

3) Hard water equivalent to 500 parts per million of calcium carbonate and composed of 0.2345 grams of calcium chloride and 0.2680 grams of magnesium chloride per liter of distilled water.

Procedure:

Three emulsion test tubes are filled with 350 milliliters of each of the test waters and placed vertically in a rack in an 85° ± 1°F

incubator.[2] The emulsifiable concentrate and test waters are brought to 85° ± 1°F and the test is conducted at this temperature through the first 24 hours. By means of syringe, an amount of concentrate equivalent to use-dilution is added through the bent needle from approximately ½ inch above the center of the test waters with a slow squeezing action. Evaluation of emulsion behaviour, referred to below, should begin at this point. After 24 hours, the emulsion test tubes are removed from the 85.0°F incubator, given one complete inversion and placed in a rack at ambient room temperature. One complete inversion consists of stoppering and inverting the emulsion tube to effect complete mixing of its contents, and then reinverting to its original vertical position. The emulsion test is evaluated again at 30, 60, 90, and 120 days.

The concentrate is then placed in a 110.0°F incubator and the test is repeated, as above at 30, 60, 90, and 120 days. Incubation at 110.0°F is intended to test the product's shelf-life, and is based on commercial shelf-life anticipation studies (4). For newly formulated emulsion concentrates, 120 days at 110.0°F is expected to simulate approximately 1.25 years of storage at ambient temperatures.

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2. An 85°F incubator may be conveniently constructed from a metal locker or wardrobe. Temperature regulation may be obtained by centrally locating a bellows-type bi-metallic thermostat actuating a microswitch attached to a line-operated hot plate. The hot plate is placed in the bottom of the locker and is insulated from the emulsion tubes by a wide wood moulding attached to the sides of the locker. This moulding provides support for the bottom of the tubes. Emulsion tubes are held by a rack made from a number of straight rubber-stamp holder strips attached to the sides of the locker. The whole cabinet should be insulated with a 2 inch blanket of

Evaluation of the Emulsion Test:

1) Initial addition of emulsion concentrate: The insecticide should disperse spontaneously in all test waters with the oil phase spreading as it sinks. No oil droplets should reach the bottom of the tube.

2) Observation at 1 hour: After standing 1 hour, there should be no more than a trace of bottom cream.

3) Observation at 24 hours: There should be no more than 0.2 ml of bottom cream in 24 hours.

4) Observation upon inversion: Any bottom cream should resuspend completely.

5) Observation at 30, 60, 90, and 120 days: There should be no more than 0.2 ml bottom cream in any of the tubes.

Repeated failure of an emulsifiable concentrate formulation to conform with the above specifications should be grounds for rejection of the product as an official permitted dip concentrate for scabies eradication. To avoid polemics, the language used to describe the evaluation of the emulsion test has been kept to a minimum. However, some controversy may still be forthcoming concerning the exact meaning of such phrases as "disperse spontaneously", "bottom cream", etc. These phrases are meant to describe real phenomena. That they do this adequately, will be immediately apparent to anyone who has had the opportunity to compare emulsion tests of products which fail the test to those which pass the test.

Other symptoms of emulsion separation such as "oiling-out", "settling",

etc. are sufficient cause for rejection of an emulsifiable concentrate as an official permitted dip. Since the emulsion must often be stable in dipping vats for extended periods, 60 days at shelf-life simulation is the minimum emulsion test observation period. For adequate safety, however, 120 days is recommended.

SPECTROPHOTOMETRIC ANALYTICAL METHODS FOR TOXAPHENE CONTENT

It has been pointed out that mere measurements of toxaphene concentration in emulsion concentrates or in vat dip samples are not to be taken as indicative, per se, of the actual or latent availability of this pesticide for acaricidal purposes. The insecticidal effectiveness of toxaphene at correct concentration is dependent largely upon emulsion particle size and emulsion stability. Therefore, a toxaphene formulation can be considered for official dipping purposes only if it will pass the emulsion stability test and if it contains the proper amount of pesticide.

The following analytical procedures are suggested to measure the requirement for toxaphene concentration in both the emulsifiable concentrate and in vat dip samples. These methods have been chosen for their accuracy, speed, and simplicity. However, the analytical laboratory is not restricted to these procedures. Although infrared spectrophotometry is believed to be more specific, this technique is not so widely available and, therefore, chemical analytical methods are also described.

Chemical assay methods for toxaphene are based upon the removal of all chlorine atoms from the molecule with subsequent recovery and titration as chloride ion. The result is then converted to toxaphene content.

Objections to chemical procedures for toxaphene reside chiefly in their non-specificity and in the intrinsic error involved: It is obvious

that any chloride obtained from other organically bound chlorine compounds, present either as contaminants or from formulation sources, will not be detected by chemical methods and will be recovered as apparent toxaphene. Further, since the chlorine content of toxaphene is 67 to 69%, an intrinsic error range of 3% obtains. This error, when taken together with the standard deviation of the chemical procedure employed, usually results in a total analytical error of ± 4 to 5%.

The infrared methods herein described obviate these errors. They are specific (18), and the standard deviation is well below ± 2%. Moreover, they are simpler and more rapid than the usual chemical analytical procedures. They should be the methods of choice for the analysis of toxaphene in emulsion concentrates and in animal vat dip samples.

Infrared Spectrophotometric Method for Emulsifiable Concentrates

This procedure was developed by F. P. Czech (10). The infrared absorption spectrum of a concentrate is taken in a short-path cell and the toxaphene content is obtained from a standard curve. Toxaphene emulsifiable concentrates should contain 61.0 ± 1.0% w toxaphene.

Since the thermal expansion coefficients for substances used in the concentrate formulation may be quite high, this test should always be conducted at the same temperature used for the establishment of the calibration curve.

Apparatus:

1) Infrared spectrophotometer, Perkin-Elmer 237, or equivalent, adapted for potentiometric linear-log

recorder, Sargent Model SRL or equivalent. The latter accessory, though optional, is useful for improving precision. The spectrum is recorded in linear absorbance on large chart paper, thereby permitting greater accuracy in reading optical density.

2) Absorption cell, fixed thickness, rock-salt windows, approximately 0.02 mm light path, Perkin -Elmer part no. 127-1190, or equivalent.

Procedure:

Preparation of calibration curve. A series of synthetic concentrates containing known amounts of toxaphene in a range from 50 to 70% should be prepared from ingredients used in the formulation under consideration. A short-path cell, about 0.02 mm in thickness, is filled with synthetic standard, and an infrared spectrum is taken at a slow scan speed (about 57 wavenumbers per minute) versus the open reference beam. The same cell is used for all determinations and a calibration curve is obtained from the series of synthetic standards as outlined below.

An absorption maximum with approximate band-center at 1303 wavenumbers (7.67 microns) is taken to be characteristic for toxaphene (Fig. 1) and obeys Lambert-Beer's law within limits (Fig. 2). Difference in absorbance, Δ A, between the absorption peak at 1303 cm^{-1} and "background" line at approximately 1420 cm^{-1} (7.04 microns) plotted against toxaphene concentration results in a useful calibration curve. (Fig. 4)

Determination of toxaphene content in a concentrate. Once a standard curve has been obtained for a proprietary concentrate, future lots of this formulation may be analyzed for toxaphene content. Moreover,

an unknown formulation may be identified or deviations from formulation may be immediately detected using the infrared technique.

The cell used for the standard curve is filled with concentrate of unknown toxaphene content and a spectrum is taken, a slow scan speed, of the appropriate spectral region. The $\Delta A_{1303 - 1420\ cm^{-1}}$ is obtained, and the amount of toxaphene of the unknown concentrate is determined directly from the calibration curve or calculated from the slope of this curve.

Results:

To date the concentrates analyzed have been free from interference from other formulation components at the two wavenumbers used in the determination. However, future formulations may require the choice of a different "background" line in order to avoid scatter or deviation from linearity. For example, the use of absorption at approximately 1850 cm^{-1} as "background" line also results in a useful though less precise calibration curve. The base-line method may also be successfully applied. Any interference at 1303 cm^{-1} will require the use of a separation technique such as described by W. H. Clark (7).

Infrared Spectrophotometric Method For Toxaphene Livestock Dip Emulsions

This procedure was developed by F. P. Czech (10). Toxaphene is "salted-out" of emulsion with sodium chloride, extracted with carbon tetrachloride, and an infrared spectrum is taken of the latter extract. Toxaphene concentration is obtained, as for the concentrate, from the difference in absorbance between the spectral background at approximately 1420 cm^{-1} and the absorption peak at 1303 cm^{-1}. Carbon tetrachloride has a relatively high thermal expansion coefficient and,

therefore, all aspects of the procedure should be conducted at the same temperature.

For acaricidal effectiveness, the concentration of toxaphene in emulsion dips should not fall below 0.50%.

Apparatus:

1) 125 ml separatory funnels with Teflon stopcock, Corning Cat. #6401, or equivalent.

2) Matched absorption cells, rock-salt windows, approximately 0.5 mm light path, Perkin-Elmer part #127-1190, or equivalent.

3) Infrared spectrophotometer equipped with potentiometric linear-log recorder as previously described.

Reagents:

1) Carbon tetrachloride, A.C.S. grade, or equivalent.

2) Sodium chloride, U.S.P. granular, or equivalent.

Procedure:

Preparation of calibration curve. Standard curves for livestock dips are prepared from appropriate aqueous dilution of an emulsion concentrate containing a known amount of toxaphene. The concentrate should be synthetically prepared from the ingredients used in the dip formulation under consideration. Alternatively, proprietary emulsion concentrates which have been subjected to thorough chemical quantitative analysis for toxaphene may be used. Standard solutions prepared from the synthetic concentrates are to be preferred in order to avoid accuracy dependent on chemical procedures.

The synthetic emulsion concentrate is diluted with distilled water to a series of concentrations ranging from 0 to 0.500 % toxaphene and

the analysis is performed as described under Analytical method.

A calibration curve is obtained, by plotting ΔA 1303 - 1420 cm^{-1} against toxaphene concentration (Fig. 3). With the parameters used, the curve is linear only below concentrations of 0.5% toxaphene.

Analytical method. A 50.00 ml aliquot of livestock dip emulsion containing up to 250 mg (0.5%) toxaphene is transferred to a 125 ml separatory funnel equipped with Teflon stopcock. To this is added 5.00 ml carbon tetrachloride and 12 to 15 g of sodium chloride. The mixture is shaken vigorously for a full 60 seconds and is allowed to stand to effect separation. The lower carbon tetrachloride layer is withdrawn and transferred to the approximately 0.5 mm path length infrared sample absorption cell. With the matched reference cell filled with carbon tetrachloride in the reference beam, a spectrum of the unknown sample is taken at a slow scan speed of approximately 57 wavenumbers per minute.

For emulsion dips containing more than 0.5% toxaphene, $\Delta A_{1303 - 1420\ cm^{-1}}$ will yield a value falling above the linear portion of the calibration curve. In such an instance, it will be desirable to extract with a larger aliquot of carbon tetrachloride.

Calculation. The difference in absorbance between 1303 cm^{-1} and 1420 cm^{-1} is related to the amount of toxaphene by use of the standard ΔA-concentration curve as described under Preparation of calibration curves. Toxaphene concentration may be read from the standard curve or calculated from the slope of this curve. Of course, any changes in extractant volume must also be considered in calculations.

Results:

Livestock dip emulsions must be subjected to a "salting-out" procedure in order to quantitatively extract toxaphene into the organic phase. Carbon tetrachloride alone will not effect complete extraction. The efficacy of combined sodium chloride "salting-out" and carbon tetrachloride extraction was checked by two independent chemical methods (2, 23) on a series of aqueous supernates. No evidence for the presence of organically bound chloride above ten micrograms/ml, the practical detection limit of the chemical methods, could be obtained. A number of different salts were tried for the purpose of "salting-out". Qualitative judgments regarding their speed and efficacy for this purpose are given in Table I.

"Salting-out" with sodium chloride results in extraction of the formulation's emulsifier and vehicle as well as toxaphene. Fortunately, the spectral nature and the relative amounts of these materials used in the proprietary emulsion concentrates investigated, is such that no interference occurs with absorption bands used for determining toxaphene. Future formulations may require the use of a different "background"line, e. g. 1850 cm^{-1}, the base-line method,or a clean-up procedure (7).

None of the large number of used animal dip solutions analyzed thus far has indicated interference from animal waste-products, contaminants, etc. Moreover, the presence of such interferences should be immediately detected by means of so powerful a qualitative technique as infrared spectrophotometry.

The results of sixteen infrared analyses on a single sample of used filth-laden livestock vat dip indicate a precision of 1.78 in terms of percent standard deviation, (Table II). Precision may be improved to some degree by reading-out absorbance with a linear-log recorder attached to the

infrared spectrophotometer.

CHEMICAL ANALYTICAL METHODS FOR TOXAPHENE CONTENT

The choice of chemical procedures for toxaphene content is left entirely to the discretion of the analyst. Procedures herein described are chosen and suggested for their simplicity, precision, speed, economy and versatility. Excellent analytical procedures for toxaphene are obtainable from Hercules Powder Co. (17). The discussion which follows is not intended as an exhaustive review of chemical analytical methods for toxaphene.

Chemical methods for chlorinated hydrocarbons, such as toxaphene, usually consist of two distinct steps. First, chlorine is quantitatively stripped from the molecule and converted to chloride ion. In the second step, the amount of chloride is determined and the result is extrapolated to toxaphene content.

Classical techniques for destroying organohalogen compounds involve tedious combustion, condensation, and/or refluxing procedures. These usually require elaborate separation operations. The recent trend is toward the use of less complex combustion procedures (31, 32), or the use of sodium metal (2,19,23) or reactive organosodium compounds (21,27,30). Only the organosodium reaction procedures require separation operations. The combustion and the sodium metal methods require adequate safety precautions, but are free from reagent deterioration, simpler, more rapid and less susceptible to error. Moreover, the final determination of chloride may be carried out in the reaction vessel.

There are many methods available for the determination of chloride obtained from the breakdown of chlorinated organic compounds. Some of the more commonly used methods are argentometric titrations using

indicators such as thiocyanate ion (Volhard, 35) chromate ion (Mohr,24) dichlorofluorescein (36), dithizone (1), variamine blue B (11); mercurimetric titrations using diphenylcarbazone as indicator (5,28,33); a mercuric hydroxycyanide method (31); colorimetric methods measuring chloranilic acid (22), or ferric thiocyanate (37), obtained through chloride ion displacements; and a variety of potentiometric (3,15,16,20) and coulometric (8,9,25) procedures.

The analytical chemist is free to use any of the above methods or any other method so long as he can obtain acceptable accuracy and reproducibility. This laboratory has employed the Cotlove automatic chloride titrator (8) primarily because of its adaptability for mass-production chloride analyses. Briefly, the instrument operates as follows: Silver ions are coulometrically generated at a constant rate and released into the titration solution where they react stoichiometrically with chloride ion. A digital read-out timer in the generator circuit begins running with the start of titration. When all chloride has reacted, the excess silver ions cause an abrupt increase in current and the titration and timer are stopped. The time required for the titration is proportional to the amount of chloride ion present. The instrument is accurate, sensitive and easy to operate.

Because of their non-specificity and the intrinsic error involved, chemical methods are not recommended for toxaphene. However, chemical methods will be useful until infrared spectrophotometry becomes a more generally employed analytical technique.

Chemical Method for the Emulsifiable Concentrate

The method described is commonly known as the Schöniger Oxygen Flask

Method (31,32)[3] and is based on the original work of W. Hempel in 1892 and others. Many modificiations, particularly in flask design, have been made since W. Schöniger's recent paper, but the principle is still the same: A sample of chlorinated hydrocarbon is burned in an atmosphere of oxygen in a specially constructed flask. The combustion products are taken up in an alkaline absorbent solution above which combustion had taken place. The amount of chloride present in acidified absorbent solution is obtained by means of the automatic chloride titrator.

Apparatus:

1) Combustion flask, Thomas-Schöniger, semimicro, 500 ml, A. H. Thomas Co., Cat. #6470-G, or equivalent.

2) Sample containers, methyl cellulose capsules, size #1, A. H. Thomas Co., Cat. #6471-G, or equivalent.

3) Weighing stand, aluminum for capsule size #1, A. H. Thomas Co., Cat. #6471-G2.

4) Automatic coulometric titrator, obtainable with accessories from American Instruments Co., Silver Spring, Maryland or Buchler Instruments, Inc., 514 West 147th St. New York 31, New York.

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3. An excellent review and exhaustive bibliography of this procedure is given by W. Schöniger in "Facts and Methods for Scientific Research", Vol, 1, No. 2, Summer 1960, published by F.& M. Scientific Corp., 1202 Arnold Avenue, New Castle County Air Base, New Castle, Delaware. Additional and more recent data may be obtained from Arthur H. Thomas Company, Philadelphia, Pennsylvania.

Reagents:

1) 0.08 N Sodium hydroxide (3.2 grams of NaOH per liter of water.)

2) 30% Hydrogen peroxide, A.C.S. grade.

3) Oxygen gas, extra dry grade, 99.6% pure, Matheson Company, or equivalent.

Procedure:

Accurately weigh approximately 40 mg of emulsifiable concentrate into a methyl cellulose capsule.[4] Alternatively, a 500 microliter volume of concentrate may be used. In this instance the specific gravity of the concentrate should be measured (cf. Calculation). Add 25.00 ml of 0.08 N sodium hydroxide absorbent solution and 2 drops (0.10 ml) of 30% hydrogen peroxide to the 500 ml combustion flask. Fill the combustion flask with oxygen. Attach a chlorine free filter paper fuse to the sample capsule and secure the capsule in the platinum sample carrier. Ignite the fuse and carry-out the combustion. CAUTION: The analyst should protect himself from the possible dangers of explosion by using gloves, safety shield, etc.

After combustion, allow the flask contents to cool, then thoroughly rinse the inside of the flask with the absorbent solution for about 3 minutes. The chloride titration may be carried out in the flask or

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4. The analyst is not restricted to a semimicro-scale procedure. If he desires, he can work with samples up to 1.5 grams (13, 14) or larger (12).

several 2.00 ml aliquots of solution may be removed for titration by the automatic chloride titrator. A sample and reagent blank or "background" should be prepared using the same amount of emulsion concentrates and diluting with reagents exactly as described above. Titrate an equivalent aliquot of "background" for chloride and subtract this from the value obtained for the unknown. The result is the net titration time in seconds, which should be used for calculating toxaphene content.

Calculations: Calculations are based on the use of the automatic chloride titrator used at the high titration rate. Calculation for toxaphene content when the sample of emulsifiable concentrate was measured by:

1) weight, $\frac{a \times t \times b \times c \times 100}{w}$ = % w/w toxaphene in concentrate

2) volume, $\frac{a \times t \times b \times c \times 100}{v \times s}$ = % w/w toxaphene in concentrate

Where:

a = titration factor in grams Cl^- / second

t = net titration time in seconds

b = aliquot of absorbing solution/aliquot used for titration = 25.1 ml/2.0 ml

c = 1.470 g of toxaphene/g of Cl^-, assuming toxaphene is 68% chlorinated

w = weight of concentrate used in grams

v = volume of concentrate used in ml

s = specific gravity of concentrate.

Chemical Method for Livestock Emulsion Dips

This method is an adaptation of the liquid anhydrous ammonia-sodium reduction procedure of H, F. Beckman, et. al. (2). Sodium reacts with

toxaphene in a diluted and dried sample of emulsion dip in liquid ammonia at about -34°C with anhydrous ethyl ether as solvent. The end of reaction is signalled by the formation of a deep-blue complex between unreacted sodium and excess ammonia. The reduction products are dried, taken up in acid and the chloride from the reaction is obtained with the automatic coulometric titrator.

Apparatus:

1) Automatic coulometric titrator, previously described.

2) Titration vial rack, wood-block with 30 holes drilled to accomodate 20 mm o.d. x 40 mm titration vials supplied with automatic titrator.

3) Glass stirring rods, Pyrex, 4 mm o.d. by approximately 80 mm in length.

4) Crystallizing dish, Pyrex glass, 6 inch diameter, mounted on cork ring (with masking tape) to isolate from warm surfaces.

Reagents:

1) Liquid anhydrous ammonia

2) Sodium metal, cut into approximately 1/8 inch cubes store under odorless kerosene.

3) Anhydrous diethyl ether, A.C.S. grade

4) Titration solution: Water plus 111 ml glacial acetic acid and 34 ml concentrated nitric acid to make 1 liter.

5) 30% Hydrogen peroxide, A.C.S. grade.

6) 2-propanol, technical, about 91%

7) Methanol + 2-propanol mixture: 2 parts methanol to 5 parts 2-propanol.

Procedure:

Thoroughly shake the sample of emulsion dip to resuspend any dirt, hair, etc., contributed by the animals. Take a 20.00 ml aliquot of dip and dilute to 100.00 ml with 2-propanol. Allow to settle and remove three 2.00 ml aliquots of clear supernate to titration vials. One of these aliquots may be titrated directly after evaporation to obtain a "background" chloride value. The other two aliquots should be evaporated to dryness at a temperature below 60°C and taken through the procedure for toxaphene content.

The reduction procedure should be carried out in a good fume hood. The analyst is advised to wear rubber gloves and safety glasses to protect himself from possible spillage or splashing of reagents and from contact with sodium.

The titration vials containing the sample evaporate are now transferred to the cork-ring mounted crystallizing dish and the dish is filled with liquid ammonia to about two-thirds the height of the titration vials. The vials should be braced to prevent tipping. Add 1 ml of anhydrous ether to each vial and allow to cool. Meanwhile, transfer a supply of sodium metal to a small beaker containing 2-propanol to effect cleaning of the surface of the sodium cubes. Add 2 ml liquid ammonia to each of the titration vials.[5]

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5. The liquid ammonia may be delivered into the reaction vial from a cylinder with a control valve to which is attached a convenient length of Teflon tubing. A stand should be contructed which permits the cylinder to be tipped for delivery. Alternatively, the cylinder may be hung in a head downward position from a convenient support. See ref. 2.

Add a cube of sodium to each vial and stir vigorously to a persistant deep blue indicating the end of reaction.

Evaporate to dryness. Add 0.2 ml of methanol:2-propanol mixture to react with any excess sodium present, and evaporate dry. Add 1 drop 30% hydrogen peroxide and again evaporate.

Take up the residue in 4 ml of titration solution and titrate for chloride with the automatic titrator.

Calculation: (Based on the use of the automatic chloride titrator at high titration rate.)

$$\frac{a \times t \times b \times c \times s_1 \times 100}{v \times s_2} = \% \text{ w/w toxaphene in dip}$$

Where:

a = instrument titration factor in grams Cl^-/second

t = net titration time

b = dilution factor = 5

c = 1.470 grams of toxaphene/gram of Cl^- assuming 68% chlorination of camphene.

s_1 = specific gravity of 20% emulsion dip in 2-propanol

s_2 = specific gravity of emulsion concentrate

v = volume of diluted sample for analysis (2 ml)

SUMMARY

The aim of this bulletin has been to present effective procedures for the evaluation of emulsifiable concentrates of toxaphene as official permitted products for livestock vat dips. The relationship between the emulsion character and the acaricidal effectiveness of a toxaphene

formulation has been discussed at length.

Physical and chemical tests are limited to those necessary to determine whether a proprietary formulation will comply with Animal Disease Eradication Division requirements. However, nothing in this bulletin implies modification of specifications and requirements which have been issued or which may be issued by regulatory offices of the Animal Disease Eradication Division or other division or agency concerned with products permitted for use in official disease eradication programs.

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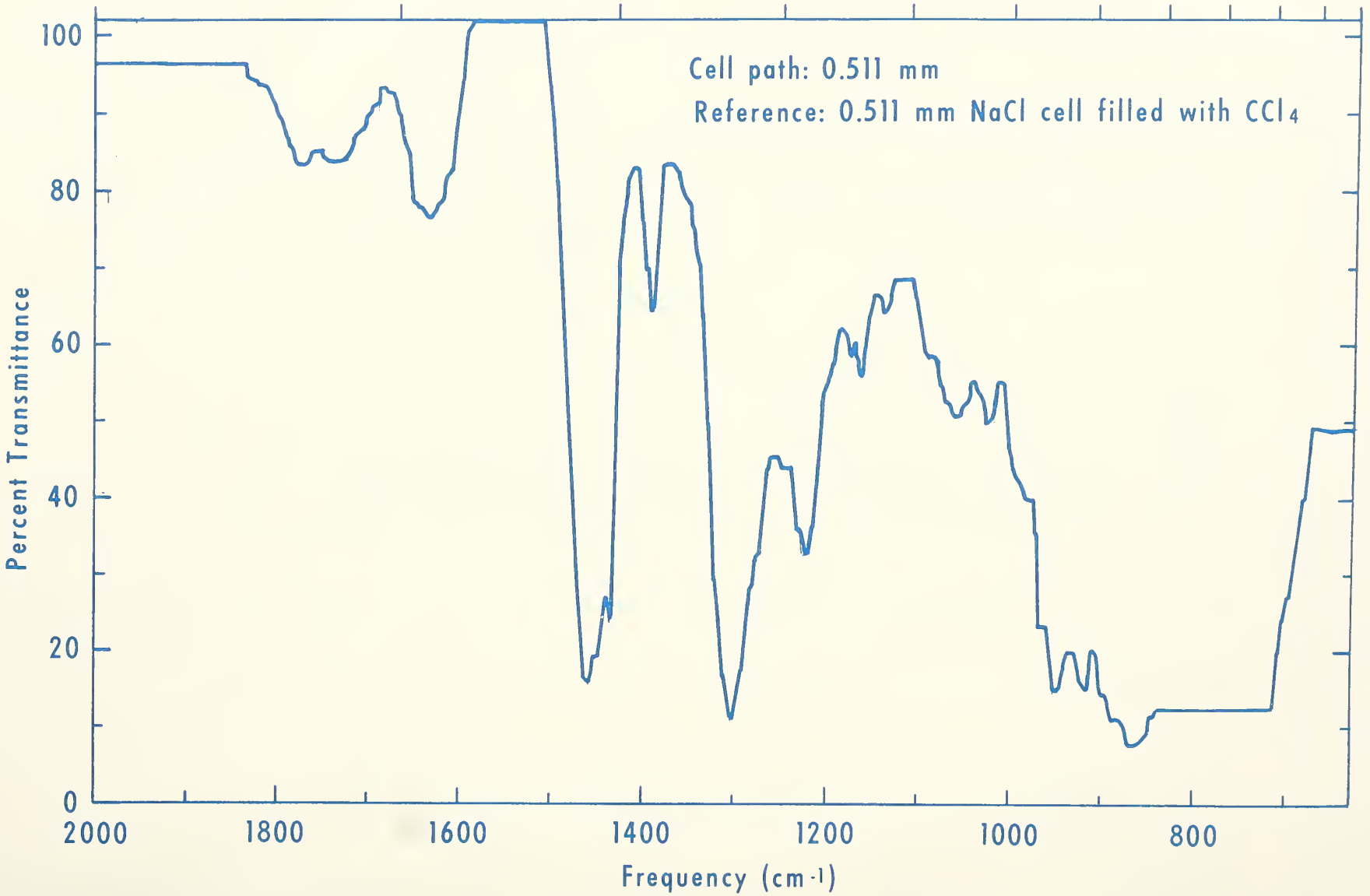
Table I: Efficiency of Some Salts Used for "Salting-out" Toxaphene from Livestock Emulsion Dips.

Salt Tried	Qualitative Judgment
NaCl	Excellent
$KHSO_4$	Good
KBr	Fair
KCl	Fair
NaBr	Fair
Na_2SO_4	Poor

TABLE II: The Precision of the Infrared Method for Livestock Toxaphene Emulsion Dips and Sprays.

$\Delta A_{1303-1420\ cm^{-1}}$	% Toxaphene (from curve)	Standard Deviation
0.414	0.5425	$\pm$ 0.0095% Toxaphene
0.409	0.5359	= 1.78% deviation
0.414	0.5425	
0.400	0.5241	
0.397	0.5202	
0.402	0.5268	
0.419	0.5490	
0.413	0.5412	
0.399	0.5228	
0.404	0.5294	
0.408	0.5346	
0.396	0.5189	
0.414	0.5425	
0.401	0.5255	
0.409	0.5359	
0.415	0.5438	

Fig. 1: (A) Infrared Spectrum of Toxaphene at 150 mg/ml in CCl_4



NOTES FIG. 1 (A & B) - - THE SPECTRUM BETWEEN c.1600 TO 1500 WAVENUMBERS HAS MOVED UPSCALE BEYOND 100% AS A COMBINED RESULT OF "DEAD PEN" ENERGY TO THERMOCOUPLE DUE TO STRONG ABSORPTION OF CCl_4 IN THIS REGION, SLIGHT UPSCALE DRIFT SETTING OF RECORDER AND SLIGHTLY GREATER AMOUNT OF CCl_4 IN REFERENCE BEAM.

Fig. 1: (B) Infrared Spectrum of Livestock Toxaphene Emulsion Dip Treated as Outlined Under Procedure

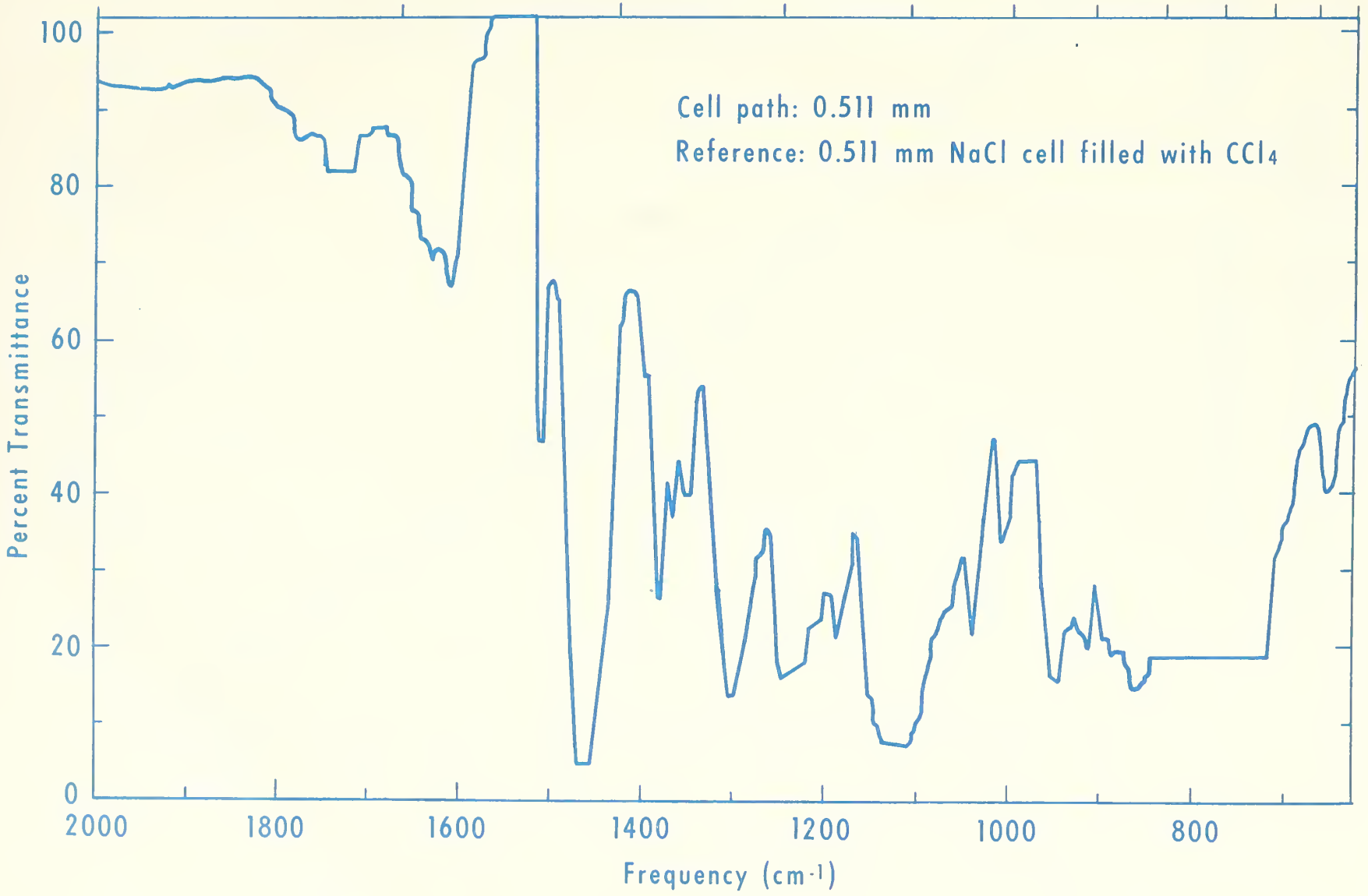


Fig. 2: Typical Calibration Curve for Toxaphene

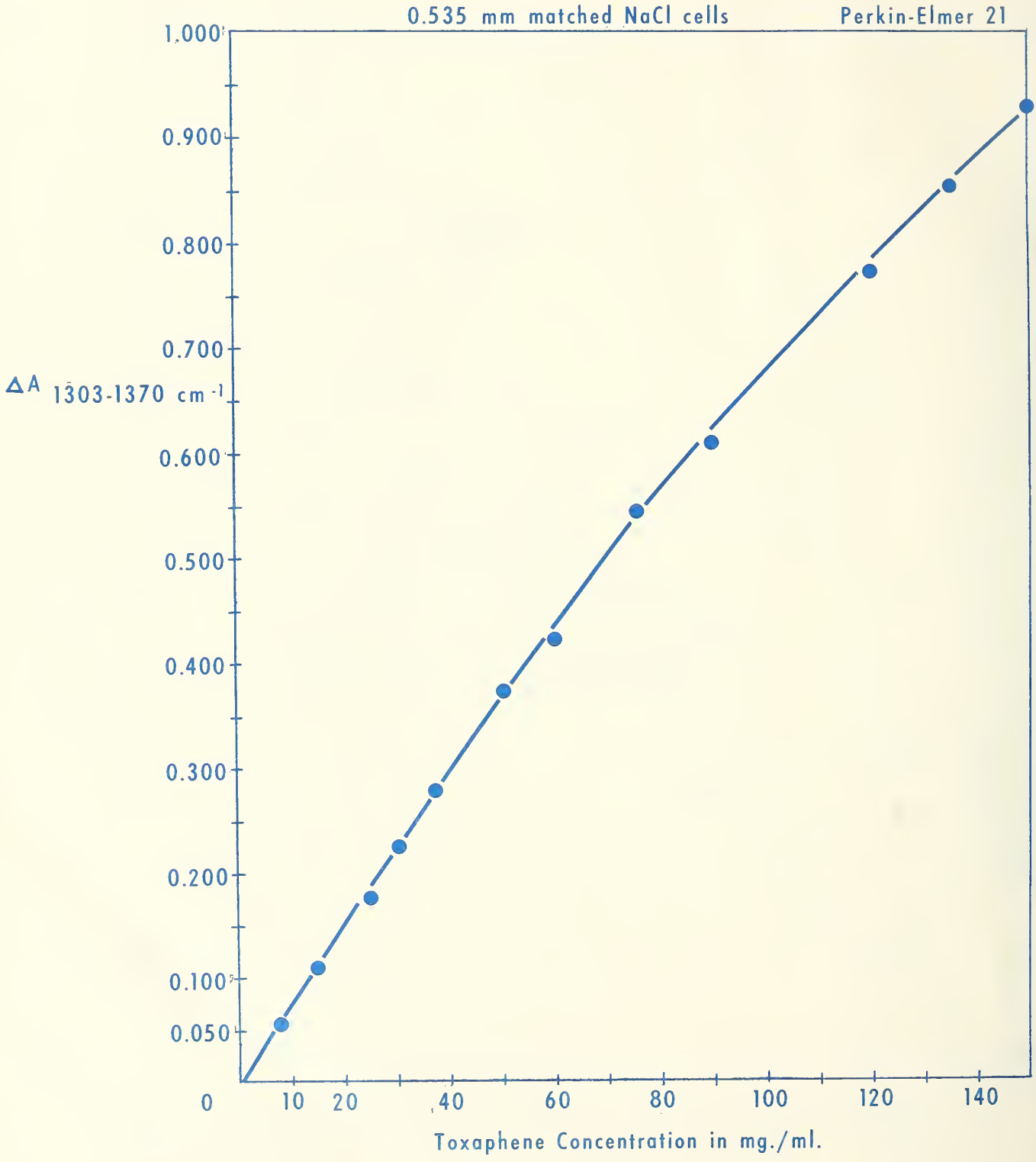


Fig. 3: Typical Calibration Curve for Toxaphene Emulsion Dips and Sprays

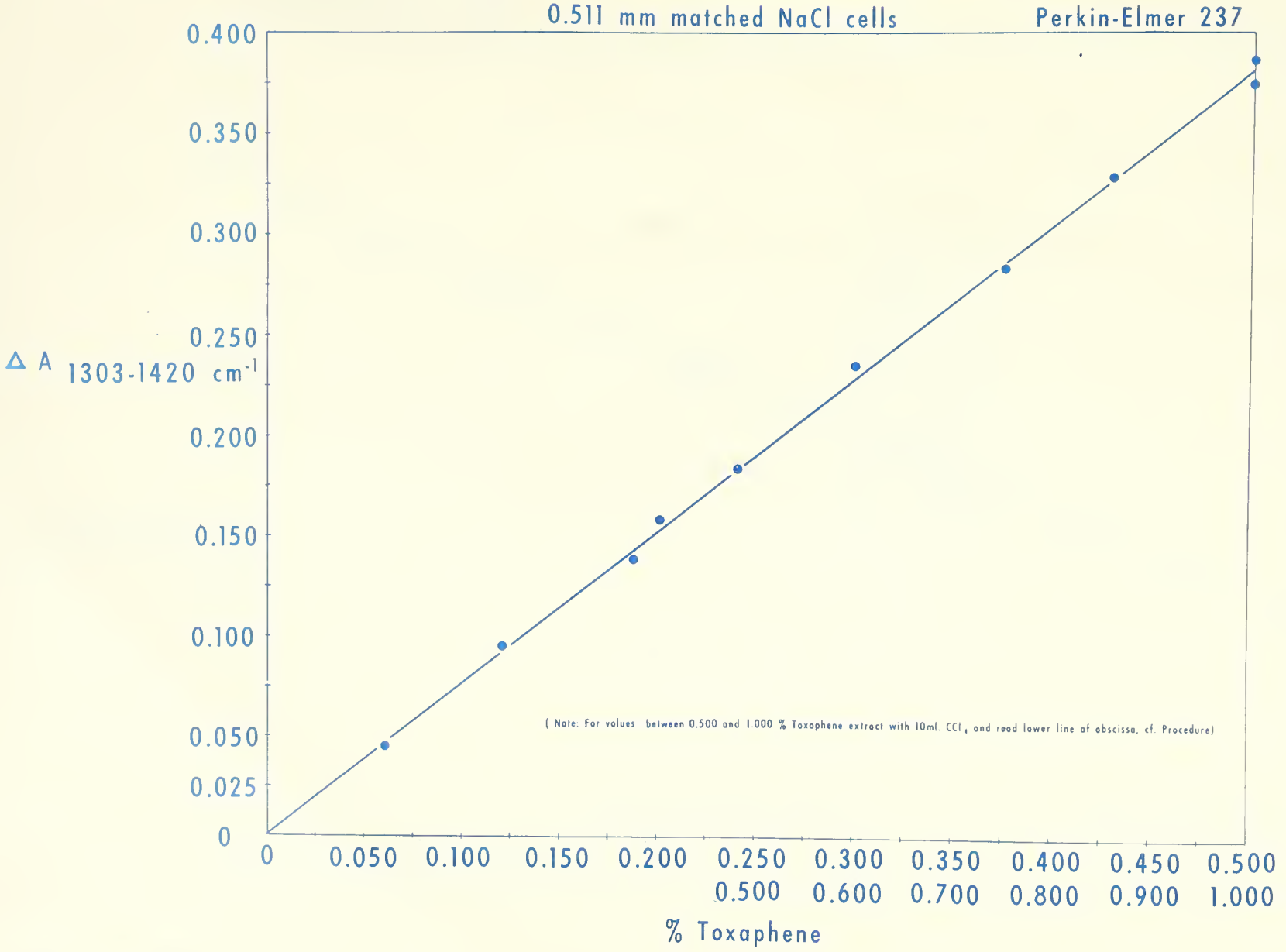
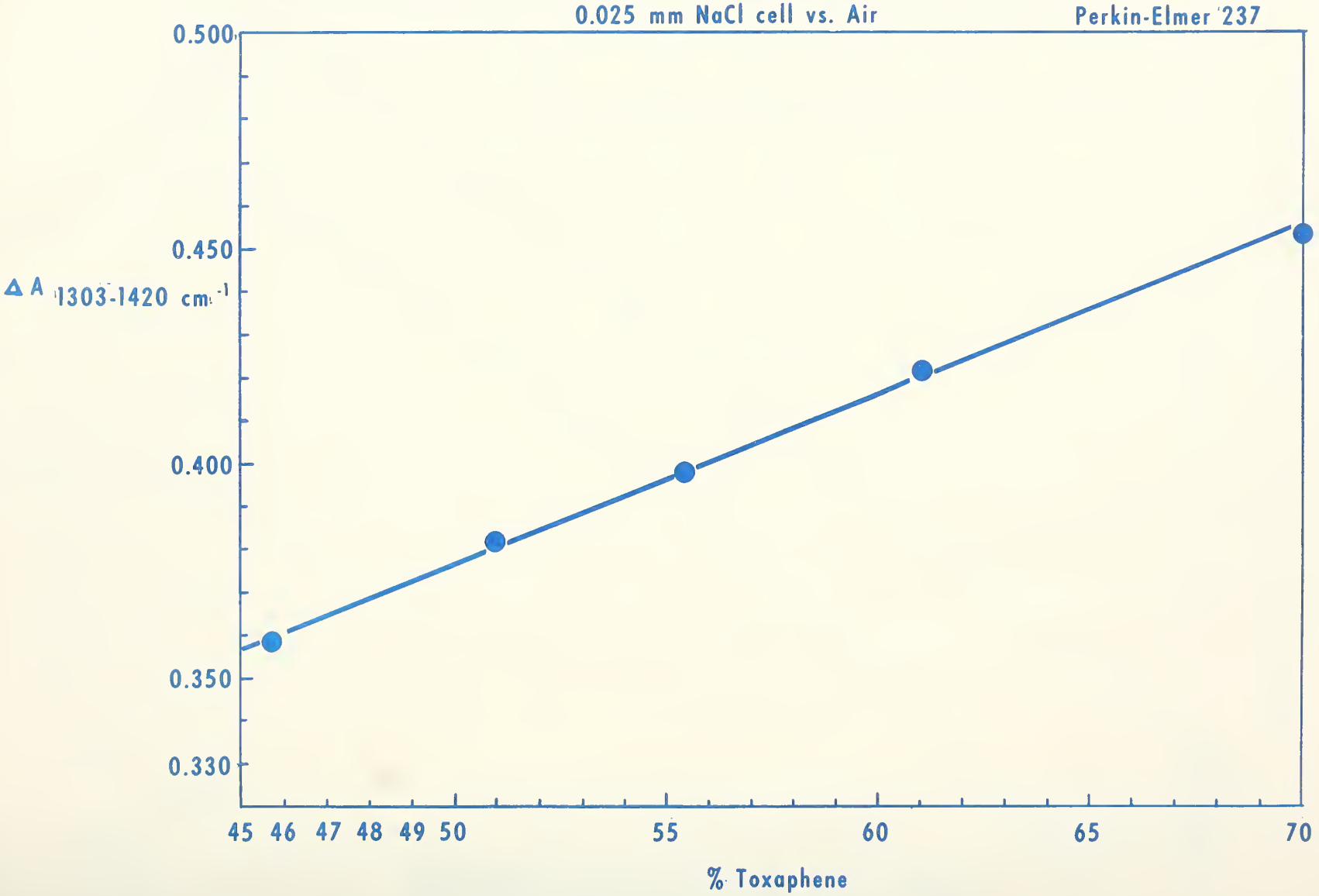


Fig. 4: Typical Calibration Curve for Toxaphene Emulsifiable Concentrates



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